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Composition and Antioxidant Activities of the Essential Oil of Cinnamon (*Cinnamomum zeylanicum* Blume) Leaves from Sri Lanka

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Abstract: The composition of the volatiles from leaves of *Cinnamomum zeylanicum* Blume from Sri Lanka was studied by GC-FID and GC-MS. The basic component of the oil was found to be eugenol (74.9%), followed by β-caryophyllene (4.1%), benzyl benzoate (3.0%), linalool (2.5%), eugenyl acetate (2.1%) and cinnamyl acetate (1.8%). The essential leaf oil from cinnamon demonstrated scavenger activity against the DPPH radical at concentrations which are lower than the concentrations of eugenol, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). This essential cinnamon oil showed also a significant inhibitory effect on hydroxyl radicals and acted as an iron chelator. Cinnamon leaf oil efficiently inhibited the formation of conjugated dienes and the generation of secondary products from lipid peroxidation at a concentration equivalent to that of the standard BHT.

Key words: *Cinnamomum zeylanicum* Blume, Lauraceae, essential leaf oil, composition of volatiles, antioxidant activities

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Introduction: *Cinnamomum zeylanicum* Blume syn. *C. verum* J. Presl, *Laurus cinnamomum* L. is an evergreen tropical tree, belonging to the Lauraceae family. Cinnamon barks and leaves are widely used as spice and flavoring agent in foods[34] and for various applications in medicine[40]. From different parts of the plant (bark, roots and leaves) essential oils with variations in the composition by especially geographic and technical reasons can be obtained. The essential oil from *C. zeylanicum* barks is rich in *trans*-cinnamaldehyde with antimicrobial effects against animal and plant pathogens, food poisoning and spoilage bacteria and fungi[6,26,29]. Until now more than 300 volatiles were found as constituents of essential oils of cinnamom[4]. The essential oil derived from cinnamom leaves is rich in eugenol, that from the roots in camphor and that from the buts shows a high amount of sesquiterpenes (α-bergamotene and α-copaene)[19]. It has been established that the oils and extracts from cinnamon possess a distinct antioxidant activity, which is especially attributed to the presence of phenolic and polyphenolic substances[8,9,20,21,25,27,31,35,39]. Free radicals are generated in consequence of the metabolism of normal or pathological cells.

The electron acceptor molecule oxygen counteracts the free radicals transforming them into a reactive oxygen species (ROS). The primary derivatives of oxygen, such as superoxide anion radicals ($O_2^{-\bullet}$), hydroxyl radicals (OH•), hydrogen peroxide (H_2O_2) and the singlet oxygen ($^1O_2$), play an important role in mediating ROS-related effects. Short-living reactive species generated *in situ* can react with non-radicals and produce chain reactions[14]. In the last years there has been a constantly rising interest in the antioxidative constituents of various medicinal plants, due to their potential in controlling the levels of free radicals and the process of lipid peroxidation[32,38].

Now, research is focused on the extraction of natural antioxidants (e.g. also from cinnamom)[33] that would be less toxic and more effective than the commonly applied synthetic antioxidants to fight the so-called "oxidative stress", which is a highly pathological situation[5]. In living organisms the protection against free radicals and reactive oxygen species is realized by antioxidative enzymes[11], reduced glutathione, vitamins, pigments, phenols and polyphenols. Synthetic antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), are well known for their ability to stop the chain reactions of lipid peroxidation.

Unfortunately, it has been shown that they are able to enhance carcinogenesis, cause pulmonary damage in mice, liver necrosis, haemorrhagic death and neoplasia in rats[17]. In this respect, flavonoids and other polyphenols are paid the greatest attention due to their non-toxicity and potentiality for implementation in human diet. Therefore, the aim of this investigation was to analyze the composition of the essential leaf oil from *Cinnamomum zeylanicum* Blume from Sri Lanka and to test the antioxidant properties of the samples and the main compound eugenol using different methods to get informations about the antioxidative activities of this phenolic monoterpene itself and the as dominating component of the essential oil of cinnamom leaves.

Experimental

Materials: The essential leaf oil of *Cinnamomum zeylanicum* Blume from Sri Lanka (800830) and eugenol are products from Kurt Kitzing Co., Wallerstein, Germany.

Chemical composition of essential oil

Gas chromatographic (GC) analysis: GC-FID analyses were carried out using a GC-14A with SPME sleeve adapted to injector, FID and C-R6A-Chromatopac integrator (Shimadzu, Japan), a GC-3700 with FID (Varian, Germany) and C-R1B-Chromatopac integrator (Shimadzu). The carrier gas was hydrogen; injector temperature, 250°C; detector temperature, 320°C. The temperature programme was: 40°C/5 min to 280°C/5 min, with a heating rate of 6°C/min. The columns were 30 m x 0.32 mm bonded FSOT-RSL-200 fused silica, with a film thickness of 0.25 μm (Biorad, Germany) and 30 m x 0.32 mm bonded Stabilwax, with a film thickness of 0.50 μm (Restek, USA). Quantification was achieved using peak area calculations, and compound identification was carried out partly using correlations between retention times[1,10,22-24].

Gas chromatographic-mass spectroscopic (GC-MS) analysis: For GC-MS measurements a GC-17A with QP5000 (Shimadzu), SPME sleeve adapted to injector and Compaq-ProLinea data system (class5k-software), a GC-HP5890 with HP5970-MSD (Hewlett-Packard, USA) and ChemStation software on a Pentium PC (Böhm, Austria), a GCQ (Finnigan-Spectronex, Germany-USA) and Gateway-2000-PS75 data system (Siemens-Nixdorf, Germany, GCQ-software) were used. The carrier gas was helium; injector temperature, 250°C; interface-heating at 300°C, ion-source-heating at 200°C, EI-mode was 70 eV, and the scan-range was 41-450 amu. For other parameters, see description of GC/FID, above. Mass spectra correlations were done using Wiley, NBS, NIST and our own library as well as published data[1,22,23].

Antioxidant activity

Scavenging effect on 2,2-diphenyl-1-picryl hydrazyl radical (DPPH): The radical scavenging ability was determined according to the method of Mensor *et al*.[30]. One ml of 0.3 mM alcoholic solution of DPPH was added to 2.5 ml of the samples with different concentration of cinnamon oil and eugenol. The samples were kept at room temperature in the dark and after 30 min the optic density was measured at 518 nm. The optic density of the samples, the control and the blank was measured in comparison with ethanol. One synthetic antioxidant, butylhydroxytoluene (BHT) and butylated hydroxyanisole (BHA) was used as positive control.

Detection of hydroxyl radicals by deoxyribose assay: The assay was performed as described by Halliwell *et al*,[15], with minor changes. All solutions were freshly prepared. 1.0 ml of the reaction mixture contained 28 mM 2-deoxy-D-ribose (dissolved in KH_2PO_4/K_2HPO_4 buffer 10 mM, pH 7.4), 500 μl solution of various concentrations of the cinnamon oil or eugenol, 200 μM $FeCl_3$ and 1.04 mM EDTA (1:1 v/v), 10 mM H_2O_2 and 1.0 mM ascorbic acid. After an incubation period of 1 h at 37^0C the extent of deoxyribose degradation was determined by the thiobarbituric acid (TBA) reaction. 1.0 ml of TBA (1 % in 50 mM NaOH) and 1.0 ml of trichloroacetic acid (TCA) were added to the reaction mixture and the tubes were heated at 100 ^{0}C for 20 min. After cooling, the absorbance was read at 532 nm against a blank (containing only buffer and deoxyribose). The percentage inhibi-

tion was calculated by the formula: $I(\%)=100 - (Abs_{sample}/Abs_{control}) \times 100$.

The IC_{50} value represented the concentration of the compounds that caused 50 % inhibition of radical formation. Quercetin was used as a positive control.

Evaluation of antioxidant activity in linoleic acid model system: Linoleic acid emulsions were prepared by mixing 0.285 g of linoleic acid, 0.289 g of Tween 20 as emulsifier and 50 ml phosphate buffer (pH 7.2). The mixture was homogenized for 5 min according to Yen *et al.*[41]. The antioxidant was added at the final concentrations of 0, 0.0025, 0.005, and 0.01 % wt/vol of oil, BHT 0.01 % was used as control. The mixture was incubated in an oven at 37^0C for 10 d. The progress of oxidation was monitored by measuring the conjugated diene formation (CD) and thiobarbituric acid reactive substances (TBARS).

The antioxidant activity at the end of assay time was expressed for each indicator as reduction percent of peroxidation (RP %) and for a control containing no antioxidant being 0%. RP % = [(peroxidation indicator value without antioxidant) – (peroxidation indicator value with antioxidant) / peroxidation indicator value without antioxidant)] x100. A higher percentage indicates a higher antioxidant activity.

Determination of conjugated dienes formation: Aliquots of 0.02 ml were taken at different intervals during incubation. After incubation, 2 ml of methanol in deionised water (60 %) were added, and the absorbance of the mixture was measured at 233 nm. The conjugated diene concentration was expressed in ml/mg in each sample. The results were calculated as CD = B x vol/wt; where B is the absorbance reading, vol denotes the volume (ml) of the sample and wt is the mass (mg) of emulsion measured[42].

Determination of thiobarbituric acid reactive substances: A modified thiobarbituric acid reactive substances (TBARS) method was used to measure the antioxidant activity of oil in terms of inhibition on lipid peroxidation. 0.1 ml of sample was taken every day, from the emulsion, the following were sequentially added: the TBA-TCA solution (20 mm TBA in 15 % TCA). The mixture was heated in a 100^0C water bath for 15 min and cooled at room temperature. After 2 ml of chloroform were added, the mixture was mixed and centrifuged at 2000 rpm for 15 min. The chloroform layer was separated and the absorbance of the supernatant was measured at 532 nm against a blank containing TBA-TCA solution. Malonic aldehyde standard curves were prepared by 1,1,3,3-tetramethoxypropane and TBARS were expressed as mg of malonic aldehyde/kg dry matter.

The data obtained at each point for all experiments were the average of three measurements.

Statistical analysis: All experimental data (in triple repetition) were included in an approximation model through polynomic dependences from fourth order. For all cases the plural correlation coefficient R^2 was determined. The level of the concentration which corresponds to 50 % of inhibition was calculated according to this approximated dependence for which R^2 is maximum. The mathematical analysis of the data is carried out with specialized MatLab (5.3/6.0) software.

Results and Discussion

Chemical composition of the essential leaf oil of *Cinnamomum zeylanicum* Blume from Sri Lanka: The essential oil of cinnamom leaves from Sri Lanka, possessing a pleasant cinnamom-leaf, eugenol- and clove-like as well as warm-floral scent, was analyzed by GC and GC-MS furnishing the following composition: Main compounds (concentrations higher than 2.0%, calculated as relative %-peak area of GC-FID analysis using an apolar column and mean-value of 3 measurements) are eugenol (74.9%), β-caryophyllene (4.1%), benzyl benzoate (3.0%), linalool (2.5%) and eugenyl acetate (2.1%). As further aroma compounds in medium concentrations (1.0-2.0%) cinnamyl acetate (1.8%), safrole (1.3%), α-pinene (1.2%) and cinnamaldehyde (1.1%) were identified. More than 20 lower concentrated volatiles are also aroma-active constituents of the *C. zeylanicum* sample (Table 1).

DPPH radical-scavenging activity: DPPH is a free radical compound that has been widely used to test the free radical-scavenging ability of various samples[16,18,36]. Figure 1 depicts the scavenging activity of cinnamon oil, eugenol, BHT and BHA on DPPH radicals at various concentrations. Cinnamon essential oil demonstrated the highest inhibitory activity compared to other antioxidants studied, reaching as high as 94.42 % at 8.0 μg/ml, while for eugenol, BHT and BHA a concentration of at 20 μg/ml was needed to achieve DPPH radicals' inhibition of 88.67, 82.40 and 89.98%, respectively. The concentration of cinnamon oil resulting in a 50 % inhibition of the free radical - IC_{50}, was 0.245 μg/ml, while for eugenol the corresponding concentration was 1.258 μg/ml. IC_{50} values were with statistical significance $p \leq 0.01$ and with high regression coefficients - $R^2 = 0.993$ (for cinnamon oil) and $R^2 = 0.999$ (for eugenol). The standards BHT and BHA showed IC_{50} of 4.407 μg/ml ($R^2 = 0.998$) and 1.12 μg/ml ($R^2 = 0.996$), respectively.

Hydroxyl radical-scavenging activity: Among all oxygen radicals the hydroxyl radical (OH•) is the most reactive and damages diverse biomolecules. Hydroxyl radicals were generated in a reaction mixture containing ascorbate, hydrogen peroxide and iron III-EDTA at pH 7.4 and measured by their ability to degrade the sugar deoxyribose[3,15]. Cinnamon oil showed high hydroxyl radical-scavenging activity that intensified with the increase of concentration, reaching 90.0% at 0.1 μg/ml, while at an equal concentration the dominant component of cinnamon oil – eugenol, caused 71.30 % inhibition of the hydroxyl radical (Figure 2). Substantially weaker was the antioxidant activity of quercetin – 77.8% at 20 μg/ml concentration. The three assayed antioxidants were arranged by their antioxidant effect (expressed as IC_{50}) in descending order, as follows: cinnamon oil - 0.046 μg/ml ($R^2 = 0.999$), eugenol - 0.055 μg/ml ($R^2 = 0.989$) and quercetin - 4.61 μg/ml ($R^2 = 0.834$). The same analytical method could also be applied for studying the inhibitory power of cinnamon oil against the metal ion-dependant generation of OH•, and not only for assaying its ability to scavenge already formed radicals.

Heavy metals, particularly those possessing two or more valency states with a suitable oxidation-reduction potential between them (e.g. Fe), increase the maximum rate of lipid oxidation. Metals act as pro-oxidants by electron transfer, liberating radicals from fatty

acids or hydroperoxides. Chelation of metal ions by food components, particularly antioxidants, reduces the pro-oxidative effect of these ions and raises considerably the energy of activation of the initiation reactions. The efficiency of cinnamon oil (in comparison with quercetin and eugenol) in reducing the pro-oxidant effect of Fe-ions by chelation was tested.

When $FeCl_3$ was added to the reaction mixture the Fe^{3+}-ions formed a complex with deoxyribose and they may be subsequently reduced by ascorbate to Fe^{2+}, which further reacts with H_2O_2. In a result, the $OH\cdot$ were generated and degradation of dexyribose occured. Only molecules that are able to chelate Fe ions and make them inactive may inhibit the degradation of deoxyribose. Results depicted on Figure 3 (without EDTA) show that cinnamon oil, eugenol and quercetin manifest chelative properties, most strongly expressed in the case of cinnamon oil.

Like most radicals, OH• can be neutralized by a hydrogen atom. The capture of OH• by cinnamon oil is attributed to the hydrogen-donating ability of the phenol eugenol, which is found in high concentrations (75.00 %) in cinnamon essential oil.

Evaluation of antioxidant activity in linoleic acid model system: For the purpose of evaluating the antioxidant activity of cinnamon oil an emulsion of linoleic acid was used as a model system. Cinnamon essensial oil was tested for its ability to inhibit the generation of hydrogenperoxides at early stages of the oxidation of linoleic acid, as well as for its inhibitory potential after the emergence of secondary oxidized products, like aldehydes, ketones or hydrocarbons. The analyses of those properties were carried out using two indicators, corresponding to the different degrees of lipid peroxidation - conjugated diene formation and TBARS.

Determination of conjugated diene formation: It was determined that the peak in conjugated diene formation was attained on the 5th day following the incubation of linoleic acid (Fig. 4A). At the higher concentration applied – 0.01%, the inhibitory effect of cinnamon oil was comparable to the action of the synthetic antioxidant BHT, namely 56.52 % for cinnamon oil and 58.69 % for BHT, measured on the 9th day of the experiment. Cinnamon oil performed weaker inhibitory effect on lipid peroxidation at 0.005% concentration – 47.82% on the 9th day of storage.

Determination of thiobarbituric acid reactive substances: With the second indicator used – TBARS, peak accumulation of malonic aldehyde was observed, as above, on the 5th day of linoleic acid storage (Fig. 4B), suggesting that the process in concern ran almost parallel to the formation of conjugated dienes. The antioxidant action of 0.01% cinnamon oil was absolutely similar to that of the standard BHT at concentration of 0.01% - the inhibition of lipid peroxidation reached 75.88% in the case of cinnamon oil and 76.47% for BHT. The lower concentration of cinnamon oil under study – 0.005%, caused insignificantly weaker inhibition of the process, reaching 71.47% on the 8th day of the experiment.

Conclusion: The essential leaf oil of *Cinnamomum zeylanicum* Blume from Sri Lanka is rich in eugenol, benzyl benzoate, linalool and eugenyl acetate as well as further

aroma-active volatiles, responsible for the pleasant cinnamom and clove-like (strong eugenol-note) and warm-floral scent of this sample[2,7,12,13,37].

The scavenging of DPPH and OH• radicals by cinnamon oil is attributed to the hydrogen-donating capacity of the phenolic component eugenol, present in significant concentrations in the essential oil. Cinnamon oil manifested higher antioxidant activity towards the DPPH radical than eugenol, BHT and BHA, confirmed by the lowest IC_{50} value. The inhibitory potential of cinnamon oil against OH• was displayed once again at a lower concentration of IC_{50} than that of eugenol and quercetin. Cinnamon essential oil demonstrated high chelative activity with respect to the Fe^{3+}, resulting in a prevention of hydroxyl radicals' initiation.

Cinnamon oil inhibited effectively the conjugated diene formation and the generation of secondary products from lipid peroxidation at concentrations equivalent to those of the standard BHT. Even more inhibited was the stage of the generation of secondary products of lipid autoxidation, i.e. cinnamon oil could also be applied as an antioxidant at a more advanced stage of lipid oxidation.

The use of some methods to determine the antioxidant properties of medicinal plant essential oils confirms the findings that antioxidant capacity detected by only a single method should be interpreted with some caution as published elsewhere[28].

In addition, our study demonstrates that cinnamon essential oil possesses considerable antioxidant capacity and could readily be implemented as a natural preservative, thus reducing or avoiding losses due to oxidative processes.

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Table 1. Chemical composition of the essential leaf oil of *Cinnamomum zeylanicum* Blume from Sri Lanka.

Compound#	%+	RI*
cis-3-Hexenol	0.1	860
Styrene	0.1	897
α-Thujene	0.2	930
α-Pinene	1.2	939
Camphene	0.3	953
Benzaldehyde	0.1	961
β-Pinene	0.3	981
Myrcene	0.1	990
α-Phellandrene	0.9	1002
cis-3-Hexenyl acetate	0.1	1005
α-Terpinene	0.1	1015
p-Cymene	0.8	1024
Limonene	0.5	1026
β-Phellandrene	0.2	1028
δ-3-Carene	0.6	1030
1,8-Cineole	0.6	1032
Benzyl alcohol	0.2	1034
trans-β-Ocimene	0.1	1049
γ-Terpinene	0.1	1059
Terpinolene	0.2	1087
Linalool	2.5	1093
Phenylethyl alcohol	0.1	1105
Benzyl acetate	0.1	1160
α-Terpineol	0.3	1188
Cinnamaldehyde	1.1	1259
Safrole	1.3	1285
α-Cubebene	0.9	1350
Eugenol	74.9	1357
Isoeugenol	0.1	1405
β-Caryophyllene	4.1	1417
Cinnamyl acetate	1.8	1444
α-Humulene	0.6	1453
α-(*E,E*)-Farnesene	1.1	1508
Eugenyl acetate	2.1	1522
trans-Nerolidol	0.1	1561
cis-3-Hexenyl benzoate	0.1	1565
Caryophyllene oxide	0.5	1581
Benzyl benzoate	3.0	1758

#in order of their retention-times, $^{+}$relative %-peak area of GC-FID analyses using an apolar column, *retention indices using an apolar OV-5-type column

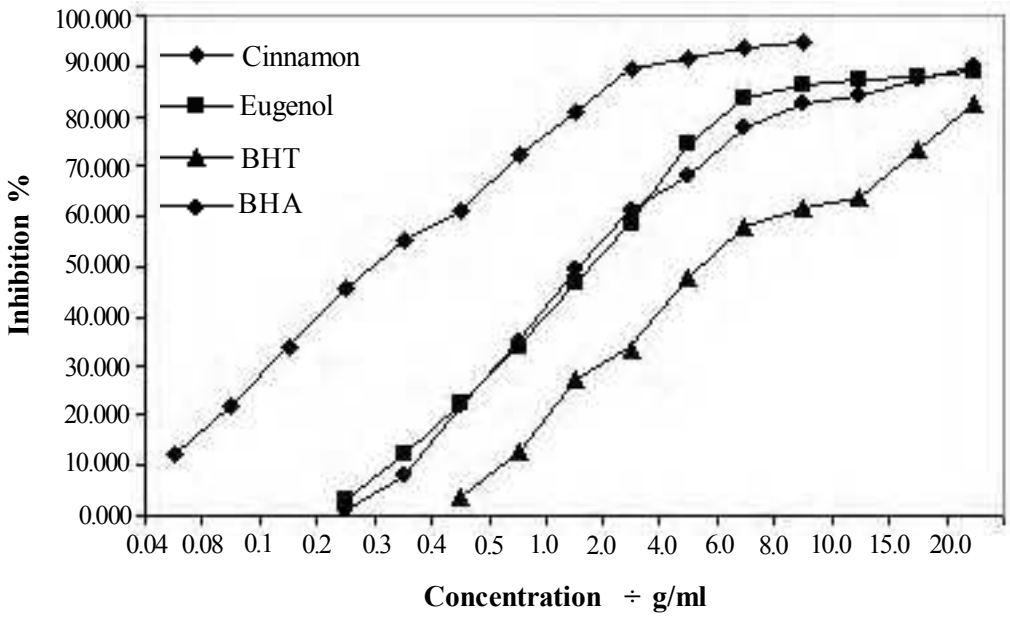


Figure 1. Scavenging effects on DPPH radical of cinnamon, eugenol, BHT and BHA

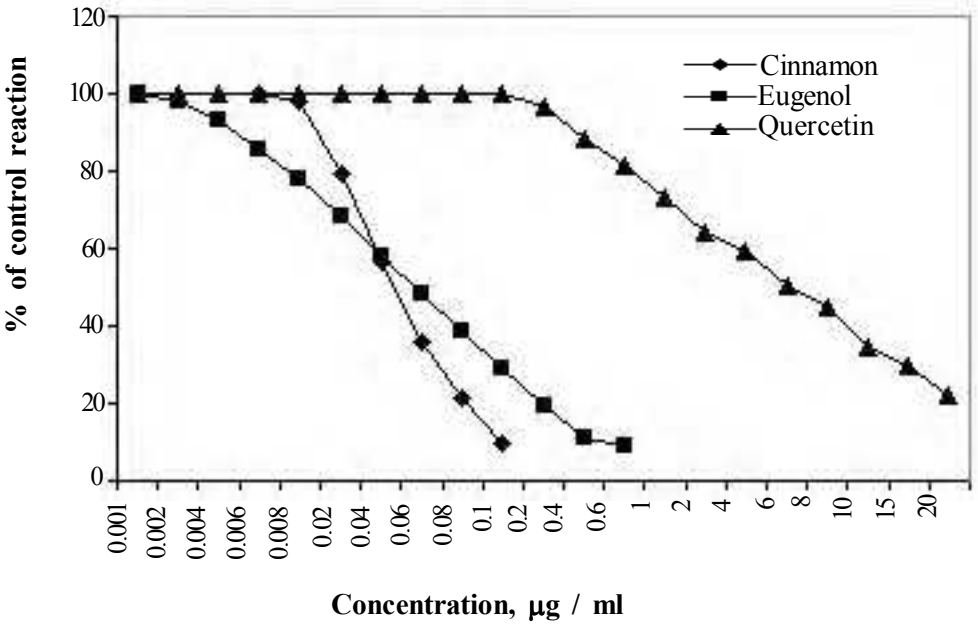


Figure 2. Action of ocinnamon oil, eugenol and quercetin on hydroxyl radical – dependent degradation of deoxyribose.

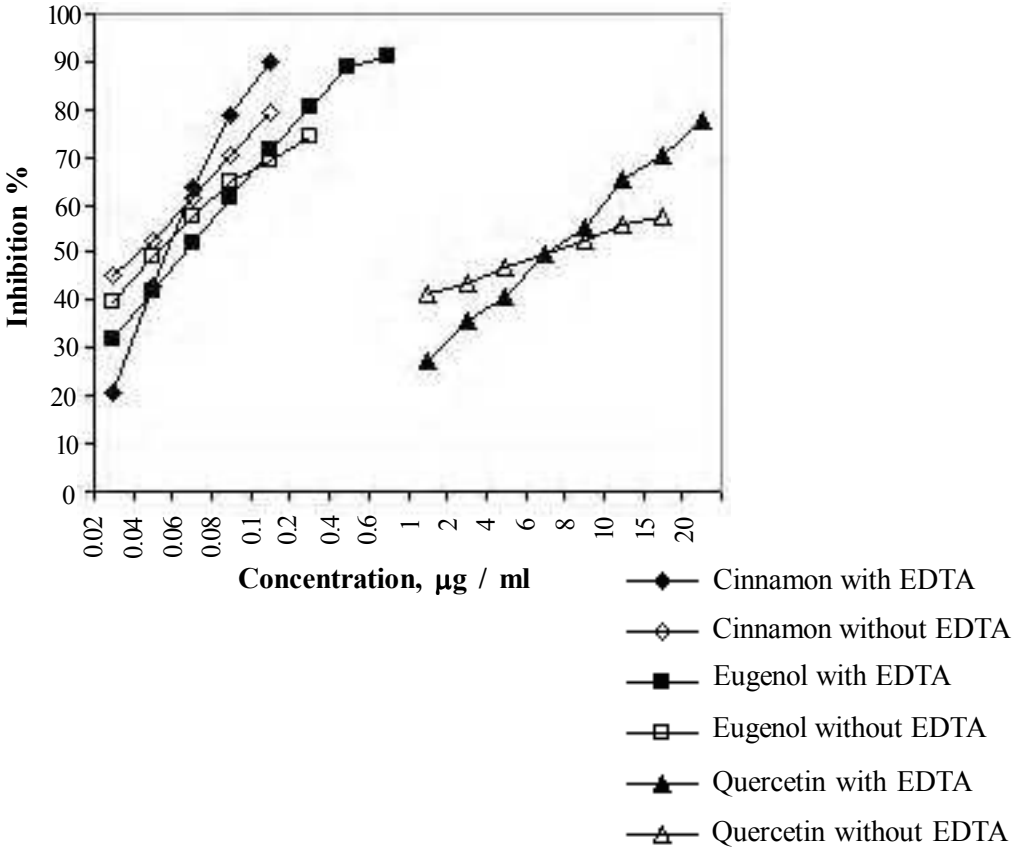
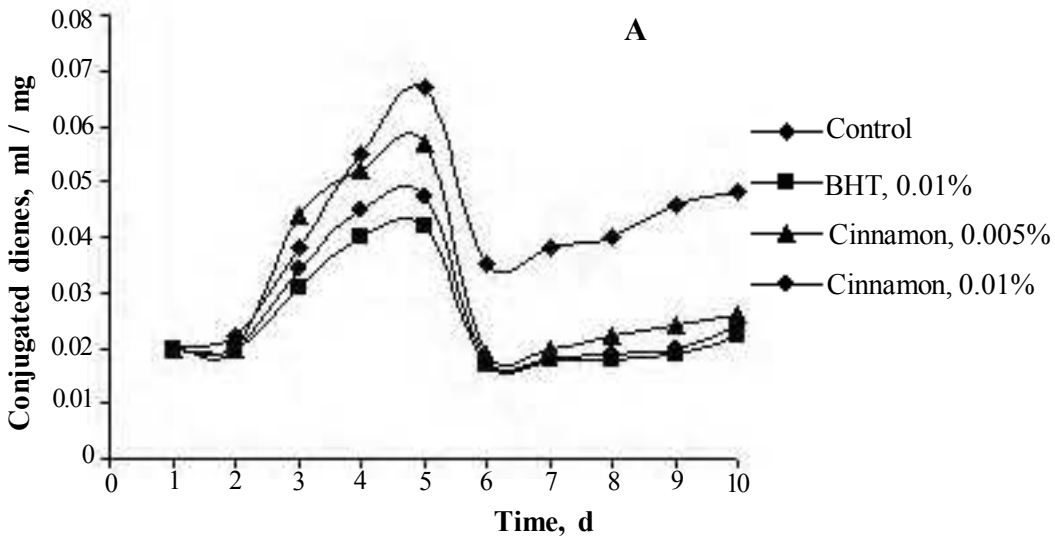


Figure 3. Metal chelating activity of cinnamon oil, eugenol and quercetin on deoxyribose degradation by OH•



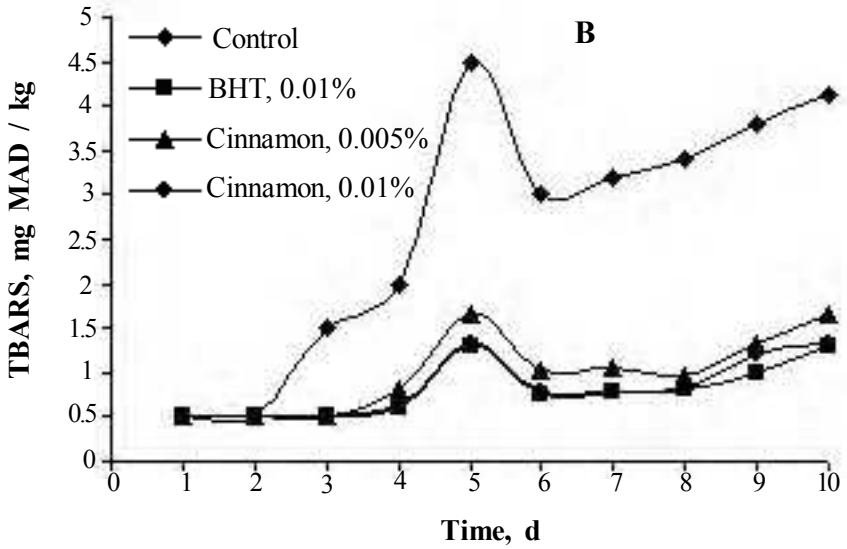


Figure 4. Effect of cinnamon oil on (A) conjugated dienes and (B) TBARS in a linoleic acid/water emulsion system